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CO_2 Laser (10.6 μm) Parameters for Caries Prevention in Dental Enamel

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Key Words
Bovine enamel · Caries prevention · CO_2 laser · Demineralisation

Abstract
Although CO_2 laser irradiation can decrease enamel demineralisation, it has still not been clarified which laser wavelength and which irradiation conditions represent the optimum parameters for application as preventive treatment. The aim of the present explorative study was to find low-fluence CO_2 laser (λ = 10.6 μm) parameters resulting in a maximum caries-preventive effect with the least thermal damage. Different laser parameters were systematically evaluated in 3 steps. In the first experiment, 5 fluences of 0.1, 0.3, 0.4, 0.5 and 0.6 J/cm^2, combined with high repetition rates and 10 μs pulse duration, were chosen for the experiments. In a second experiment, the influence of different pulse durations (5, 10, 20, 30 and 50 μs) on the demineralisation of dental enamel was assessed. Finally, 3 different irradiation times (2, 5 and 9 s) were tested in a third experiment. In total, 276 bovine enamel blocks were used for the experiments. An 8-day pH-cycling regime was performed after the laser treatment. Demineralisation was assessed by lesion depth measurements with a polarised light microscope, and morphological changes were assessed with a scanning electron microscope. Irradiation with 0.3 J/cm^2, 5 μs, 226 Hz for 9 s (2,036 overlapping pulses) increased caries resistance by up to 81% compared to the control and was even significantly better than fluoride application (25%, $p < 0.0001$). Scanning electron microscopy examination did not reveal any obvious damage caused by the laser irradiation.

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The possibility of making dental enamel more resistant to caries attack by irradiation with a CO_2 laser has been described for all its main wavelengths [Featherstone et al., 1998]. The 9.6-μm wavelength has 10 times higher absorption in enamel (8,000 cm^{-1}) than the 10.6-μm wavelength (825 cm^{-1}) and has therefore been considered the most promising for use in caries prevention [Zuerlein et al., 1999]. However, the lower absorption of the 10.6-μm wavelength results in a higher penetration depth and can therefore affect a thicker enamel layer. For this reason, it has been suggested that the caries-preventive effect obtained with 10.6 μm could be longer-lasting [Fox et al., 1992]. Furthermore, the most recent studies have demonstrated that the irradiation of dentin with a 9.3-μm CO_2 laser failed to show any statistically significant increase in acid resistance [Le et al., 2008], whereas a continuous-wave 10.6-μm CO_2 laser was able to decrease the acid dissolution rate at higher power settings [Featherstone et al., 2008]. Therefore, it may be possible that the 10.6-μm

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Table 1. Description of the CO_2 laser parameters used to investigate the influence of energy density on the caries-preventive effect (experiment 1)

Parameters	Groups				
	0.1 J/cm^2	0.3 J/cm^2	0.4 J/cm^2	0.5 J/cm^2	0.6 J/cm^2
Pulse duration, μs	10	10	10	10	10
Average power, W	3	2.2	3.2	4.5	5.3
Repetition rate, Hz	500	154	167	182	186
Pulse energy, mJ	6	14	19	25	29
Irradiation time, s	5	15	5	2	1
Number of pulses	2,500	2,308	833	364	186
Duty cycle, %	0.5	0.15	0.16	0.18	0.19
Beam diameter, mm	2.5	2.5	2.5	2.5	2.5
Beam profile	TEM_{00}	TEM_{00}	TEM_{00}	TEM_{00}	TEM_{00}

Table 2. Description of the CO_2 laser parameters used to investigate the influence of pulse duration on the caries-preventive effect (experiment 2)

Parameters	Groups				
	5 μs	10 μs	20 μs	30 μs	50 μs
Energy density, J/cm^2			0.3		
Average power, W	3.2	2.3	1.4	2.3	2.7
Repetition rate, Hz	226	154	102	111	70
Pulse energy, mJ	14	14	14	21	39
Irradiation time, s	9	15	19	10	15
Number of pulses	2,036	2,308	1,939	1,114	1,050
Duty cycle, %	0.11	0.15	0.20	0.33	0.35
Beam diameter, mm	2.5	2.5	2.5	3	4.5
Beam profile	TEM_{00}	TEM_{00}	TEM_{00}	TEM_{00}	TEM_{00}

wavelength produces the more balanced caries-preventive effect in all dental hard tissues.

Greater penetration of the light naturally increases the risk of higher temperatures in deeper tissue layers. However, recent technical improvements allow the use of very short pulse durations below the thermal relaxation time of enamel (<90 μs). For the same fluence, the reduction of the pulse duration from 500 to 50 μs causes a difference of 200°C in the enamel surface temperature increase. The shorter pulses lead to a higher surface temperature, but with lower heat propagation to the deeper layers, thus decreasing the risk of pulpal damage [Fried et al., 1996]. In addition, reduction of the pulse duration from 100 to 2 μs halves the amount of energy needed to eliminate the carbonate from the enamel structure [Fried et al., 1999].

Here, we describe a systematic evaluation of 10.6-μm low-fluence CO_2 laser parameters for application in dental enamel.

Materials and Methods

Samples

In total, 270 bovine enamel blocks (4 × 4 × 3 mm) were obtained from bovine incisors and stored in 0.1% thymol solution, pH 7, at 4°C after extraction [ten Cate et al., 2006]. The buccal surfaces of the blocks were flattened and polished with No. 800 and No. 4000 Al_2O_3 abrasive papers, followed by 30-second sonication baths. The blocks were examined under a stereoscopic microscope, and those presenting cracks or any structural defects were discarded. The remaining samples were covered with acid-resistant varnish, except for a round window of 2.5 mm diameter on the buccal side, and then randomly allocated to the treatment groups.

Laser Irradiation Conditions

Irradiation was performed using a CO_2 laser emitting at 10.6 μm (model Rofin SCx 30, Rofin-Sinar Laser GmbH, Hamburg, Germany). In order to allow adequate determination of the energy densities, and considering that the laser beam had gaussian distribution and radial symmetry, the beam diameter at $1/e^2$ of the intensity level was determined using the knife edge method. The emitted energy was controlled using an energy meter (Coherent Field Master GS + Detector LM45; Coherent, USA). Irradiation was performed at a distance of 19.8 cm, and the beam diameter at the sample surface was 2.5 mm.

In a first experiment, 5 groups (n = 15) were irradiated with 5 different energy densities of 0.1, 0.3, 0.4, 0.5 and 0.6 J/cm^2. A non-irradiated group was prepared as a control. The complete irradiation parameters can be taken from table 1. After determining the energy density that resulted in the lowest demineralisation, the samples were irradiated with different pulse durations ranging from 5 to 50 μs in a second experiment (table 2). A non-irradiated group served as a negative control and an additional group, which was treated by acidulated phosphate fluoride gel application, served as a positive control. The acidulated phosphate fluoride gel (DFL Ltd., Rio de Janeiro, Brazil) contained 1.23% fluoride, had a pH value of 3.5 and was applied for 4 min. In this experiment, the beam diameter of 2 groups (groups 30 and 50 μs, 3 and 4 mm diameter, respectively) was changed in order to maintain the same energy density in all groups. In a final experiment, based on the optimum combination of energy density and pulse duration, the influence of different irradiation times on the demineralisation of the samples was investigated. Demineralisation in samples irradiated for 2, 5 and 9 s, which had 452, 1,130 and 2,036 overlapping pulses, respectively, was compared with non-irradiated and fluoride-treated samples. All the other irradiation parameters were the same for all the experimental groups and were the ones

used for the irradiation of the 5-μs pulse duration group in experiment 2.

pH Cycling

After irradiation, all samples were subjected to a pH-cycling model, which is a modification of an earlier proposed one [ten Cate and Duijsters, 1982] and that was described by Queiroz et al. [2008]. The samples were submitted to the following protocol during 9 days (8 + 1 day remineralisation bath) at 37°C: (1) 4 h in 31 ml demineralisation bath (1.28 mM calcium nitrate, 0.74 mM sodium dihydrogen phosphate, 0.05 M acetate buffer, 0.03 μg F/ml, pH 5.0); (2) the specimens were individually rinsed thoroughly for 10 s in distilled water and dried carefully with absorbent paper in order to avoid dilution of the individual baths; (3) 20 h in 15 ml remineralisation bath (1.5 mM calcium nitrate, 0.9 mM sodium dihydrogen phosphate, 150 mM potassium chloride, 0.1 M Tris buffer, 0.05 μg F/ml, pH 7); (4) after 8 days of cycling, the blocks remained in the remineralisation solution for 24 h.

The plastic bottles containing the samples were maintained at 37°C and under constant agitation at 200 rpm during the whole cycling period [Queiroz et al., 2008]. After completion of the cycling procedure, before and between the further investigations, the specimens were stored on wet cotton fabric at room temperature and a constant relative humidity of 100%.

Lesion Depth Measurements

The samples were sectioned through the centre of the lesions using a diamond band saw with water cooling (model E 300, Exakt GmbH, Norderstedt, Germany). One of the halves was serially dehydrated in alcohol (70, 80, 90, 96 and 100%) and then embedded in acrylic resin for histological sections (K-Plast, Medim Histotechnologie GmbH, Giessen, Germany). The resin blocks were cut into slices with a thickness of approximately 300 μm (Exakt GmbH) and subsequently ground and polished to a thickness of 100 μm (±10 μm) [Apel et al., 2003].

After immersion in distilled water, the specimens were examined under a transmitted-light microscope equipped with a digital camera (DM-R-HR, polarisation filter, lambda filter, Leica GmbH, Bensheim, Germany). The images were obtained using a ×10 magnification objective, and the measurements of the demineralisation depth zones were performed by an examiner blinded to the test groups using the software Diskus 4.20 (Hilgers/Leica GmbH, Bensheim, Germany). As the laser effects on the tissue may be affected by the gaussian profile of the energy distribution inside the beam, the lesion depth measurements were performed in 3 different sectors [Meister et al., 2003]. The outer, intermediate and inner sectors were located at a distance of 0–400, 400–800 and 800–1,200 μm from the lesion margin, respectively, and 6 measurements were performed in each of them.

Scanning Electron Microscopy

Morphological investigations were performed in order to verify the effects of irradiation at the enamel surface. Only the irradiation conditions causing the greatest caries inhibition in each experimental step were chosen for this. Two additional samples from each group were irradiated and a further 2 not treated, these serving as a control. The irradiated samples were then serially dehydrated in alcohol, immersed in hexamethyldisilazane for further dehydration, covered with a thin gold layer and examined under an environmental scanning electron microscope (ESEM XL30 Field Emission Gun, Phillips, Eindhoven, the Netherlands). The images were obtained using a gaseous secondary electron detector, the sample's chamber pressure was around 1 mbar, the accelerating voltage 20 kV and the working distance 10 mm.

For the cross-sectional observation of the irradiated samples, 2 other samples were used. Prior to the dehydration in alcohol, a deep slit was made in their back surface with a diamond disc, in order to facilitate the subsequent cryofracture. After alcohol dehydration, the samples were immersed for 5 min in liquid nitrogen and fractured along the preformed slits. The samples which did not fracture spontaneously were gently split with a hammer and a scalpel blade.

Statistical Analysis

Lesions were summarized as mean and standard deviation, separately for each group, each sector or each combination of these two factors.

For each of the 3 experiments, a nested effects linear model with interaction was fitted to the obtained lesion depth measurements. This model allows analysis of the main effects of 'group', 'block' (nested within 'group') and 'sector' (nested within 'block'), with 6 repeated measurements at each sector, as well as the group × sector interaction effect on lesion depth. In this model, the nested factor 'sector' has 3 different levels (inner, intermediate and outer) while the factor 'group' has 6, 7 and 5 different levels in experiments 1, 2 and 3, respectively. Because blocks were allocated randomly to treatment groups, we modelled the nested effect of block (within group) as random effect.

A global statistical significance level of $\alpha = 5\%$ was chosen for all statistical analyses conducted. Because of the explorative character of this series of experiments, the test results yielded in all statistical test procedures carried out were interpreted in an explorative manner only; thus, p values <0.05 were regarded as statistically significant test results (with respect to the investigated sample of dental enamel blocks).

Depending on the significance results yielded in the global F tests conducted according to each of the main and interaction effects incorporated in the nested effects model, different post-hoc multiple pairwise comparisons were carried out. If the group × sector interaction effect turned out to be statistically significant, post hoc unpaired t tests were performed in order to compare the treatment groups in pairwise fashion within each of the 3 sectors (averaging over the 6 different measurements made within each group). Additionally, post hoc paired t tests were carried out to compare the 3 sectors in pairwise fashion within each of the treatment groups (again by averaging over the 6 different measurements made within each sector). When the group × sector interaction effect did not prove to be statistically significant, in contrast to the main effect of group, post hoc unpaired t tests were conducted for global pairwise comparison of treatment groups (averaging over sectors and measurements). All statistical analyses were performed by use of the SAS statistical software package, version 9.1 (SAS for Windows, SAS Institute Inc., Cary, USA).

Table 3. Observed mean lesion depths (μm) and standard deviations (SD) in the different lesion sectors (outer, intermediate and inner) for all groups from the investigation of the influence of energy density on the caries-preventive effect (experiment 1)

Groups	n	Code	Outer (A)			Intermediate (B)			Inner (C)		
			mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors
Laser 0.1 J/cm^2	13	a	46 ± 12	b, c, d, e, f	B, C	24 ± 12	b, e, f	A, C	14 ± 6	c, d, e, f	A, B
Laser 0.3 J/cm^2	10	b	32 ± 11	a, c, d, e, f	B, C	19 ± 9	a, c, d, e, f	A, C	16 ± 9	c, d, e, f	A, B
Laser 0.4 J/cm^2	14	c	34 ± 13	a, b, d, e, f	B, C	25 ± 13	b, e, f	A, C	21 ± 14	a, b, f	A, B
Laser 0.5 J/cm^2	14	d	37 ± 13	a, b, c, e, f	B, C	26 ± 9	b, e, f	A, C	21 ± 7	a, b, f	A, B
Laser 0.6 J/cm^2	13	e	45 ± 9	a, b, c, d, f	B, C	28 ± 10	a, b, c, d, f	A, C	20 ± 8	a, b, f	A, B
Control	13	f	52 ± 8	a, b, c, d, e	C	52 ± 7	a, b, c, d, e	C	49 ± 8	a, b, c, d, e	A, B

Statistical difference between the groups in one sector is represented by different lower-case letters, and between the sectors for one group by different capital letters ($\alpha = 0.05$).

Table 4. Observed mean lesion depths (μm), standard deviations (SD) and percentage caries inhibition in relation to the control in the experimental step investigating the different pulse durations (experiment 2)

Groups	n	Code	Outer (A)			Intermediate (B)			Inner (C)			All sectors, mean ± SD	Inhibition of caries progression, %
			mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors		
Laser 5 μs	15	a	26 ± 10	c, d, e, f, g	B, C	21 ± 9	b, c, d, e, f, g	A, C	14 ± 7	b, c, d, e, f, g	A, B	20 ± 10	69
Laser 10 μs	14	b	27 ± 10	c, d, e, f, g	C	26 ± 11	a, c, d, e, f, g	C	21 ± 10	a, c, d, e, f, g	A, B	25 ± 13	54
Laser 20 μs	15	c	53 ± 13	a, b, d, e, f, g	n.s.	54 ± 11	a, b, e, f, g	C	52 ± 10	a, b, d, e, f, g	B	53 ± 12	11
Laser 30 μs	15	d	47 ± 14	a, b, c, e, f, g	B, C	53 ± 17	a, b, e, f, g	A, C	51 ± 16	a, b, c, e, f, g	A, B	50 ± 16	20
Laser 50 μs	14	e	56 ± 12	a, b, c, d, f, g	B, C	59 ± 14	a, b, c, d, f, g	A	59 ± 15	a, b, c, d, f, g	A	58 ± 14	6
Control	15	f	59 ± 15	a, b, c, d, e, g	B, C	66 ± 15	a, b, c, d, e, g	A, C	69 ± 16	a, b, c, d, e, g	A, B	65 ± 16	0
Fluoride	14	g	33 ± 27	a, b, c, d, e, f	n.s.	34 ± 12	a, b, c, d, e, f	n.s.	36 ± 13	a, b, c, d, e, f	n.s.	34 ± 19	44

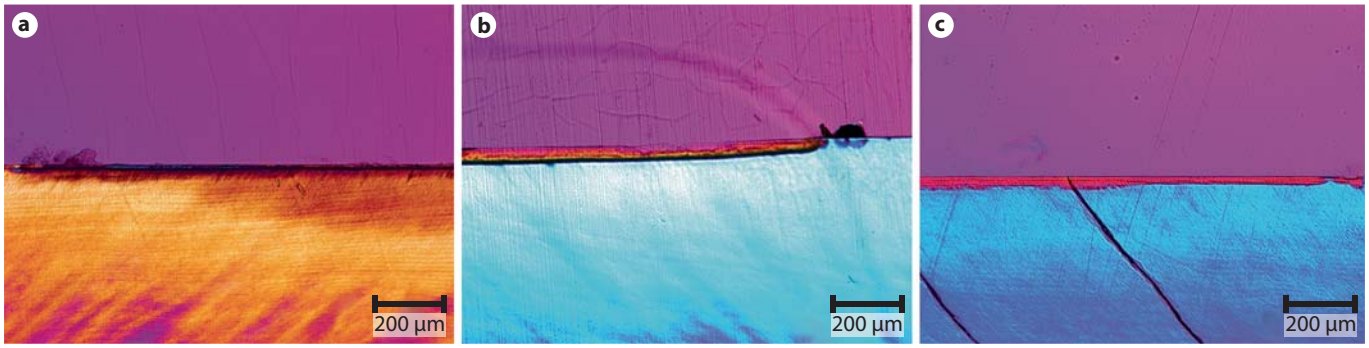
For explanations, see table 3. n.s. = None of the corresponding pairwise comparisons was statistically significant.

Results

Lesion Depth Measurements

In experiment 1, the results of the statistical analysis showed that the group irradiated with 0.3 J/cm^2 presented the lowest means in outer and intermediate lesion sectors (32 ± 11 and 19 ± 9 μm) and the group irradiated with 0.1 J/cm^2 the lowest in the inner sector (14 ± 6 μm). Nevertheless, the irradiation with 0.3 J/cm^2 resulted in significantly lower means than the control and all the other laser groups in all 3 sectors ($p < 0.0001$). Therefore, the energy density of this group was maintained for the next experimental step (experiment 2). The analysis also showed that there were significant differences between the groups ($p < 0.001$), between the sectors ($p < 0.001$) and also in the interaction between groups and sectors ($p < 0.001$). For all the laser-treated groups, the means of the lesion depth were significantly lower in the intermediate and inner than in the outer sector (table 3).

In experiment 2, the group irradiated with 5 μs of pulse duration presented the lowest means and the control group the highest (table 4). ANOVA revealed that there was a statistically significant difference between the groups ($p < 0.001$) and between the sectors ($p < 0.001$). The interaction of groups and sectors was also significant ($p < 0.001$). In all 3 sectors, the mean lesion depth of the fluoride-treated group was significantly lower than that of the control group (all $p < 0.001$), and the group irradiated with 5 μs of pulse duration presented means significantly lower than both control (all $p < 0.001$) and fluo-



Color version available online

Fig. 1. Polarised light microscope pictures of enamel samples after pH cycling. **a** Group laser 9 s (0.3 J/cm^2, 5 µs, 226 Hz) showing shallow lesion formation below the irradiated surface. **b** Untreated control group revealing deeper lesion. **c** Fluoride-treated sample of the positive control group showing an intermediate lesion depth.

Table 5. Observed mean lesion depths (µm), standard deviations (SD) and percentage caries inhibition in relation to the control, considering the irradiation time (experiment 3)

Groups	n	Code	Outer			Intermediate			Inner			All sectors, mean ± SD	Inhibition of caries progression, %
			mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors	mean ± SD	between groups	between sectors		
Laser 2 s	15	a	20 ± 8	b, c, d, e	C	20 ± 9	b, c, d, e	C	18 ± 10	b, c, d, e	A, B	20 ± 9	32
Laser 5 s	12	b	13 ± 5	a, c, d, e	B, C	10 ± 4	a, c, d, e	A, C	9 ± 3	a, c, d, e	A, B	10 ± 5	63
Laser 9 s	13	c	6 ± 4	a, b, d, e	C	6 ± 3	a, b, d, e	C	5 ± 2	a, b, d, e	A, B	6 ± 3	81
Control	14	d	28 ± 7	a, b, c, e	B, C	30 ± 8	a, b, c, e	A	30 ± 6	a, b, c, e	A	29 ± 7	0
Fluoride	15	e	22 ± 6	a, b, c, d	n.s.	21 ± 6	a, b, c, d	n.s.	22 ± 7	a, b, c, d	n.s.	22 ± 6	25

For explanations, see table 3. n.s. = None of the corresponding pairwise comparisons was statistically significant.

ride-treated groups (all $p < 0.001$). Besides, the group irradiated with 5 µs had means statistically significantly lower than the one irradiated with 10 µs in 2 lesion sectors ($p = 0.079$, $p < 0.001$, $p < 0.001$). Therefore, the conditions of this group were maintained for experiment 3.

In experiment 3, the group irradiated for 9 s presented the lowest means (table 5), which were statistically significantly lower than those of all the other groups, including the control group (all $p < 0.001$) and the fluoride-treated group (all $p < 0.0001$ – table 5 and fig. 1). The results also revealed that there was a statistically significant difference between the groups ($p < 0.001$), between the sectors ($p < 0.001$), and there was significance in the interaction between groups and sectors ($p < 0.001$).

During the polishing procedures a total of 22 of the 270 samples were lost, and the final numbers used for the statistical analysis are shown in tables 3–5.

Scanning Electron Microscopy

Scanning electron microscopy revealed that the irradiated surfaces in all groups had a similar appearance to the non-irradiated controls. Moreover, no signs of ablation, melting or surface damage, such as cracks or fissures, could be observed. For the parameters showing the greatest caries inhibition (0.3 J/cm^2, 5 µs, 226 Hz, 2,036 overlapping pulses and 9 s of irradiation), cross-sectional images showed that no damage was observable even in subsurface regions (fig. 2).

Discussion

After systematic evaluations of different CO_2 laser ($\lambda = 10.6$ µm) parameters for enamel caries prevention, we were able to find a set of parameters yielding 81% dis-

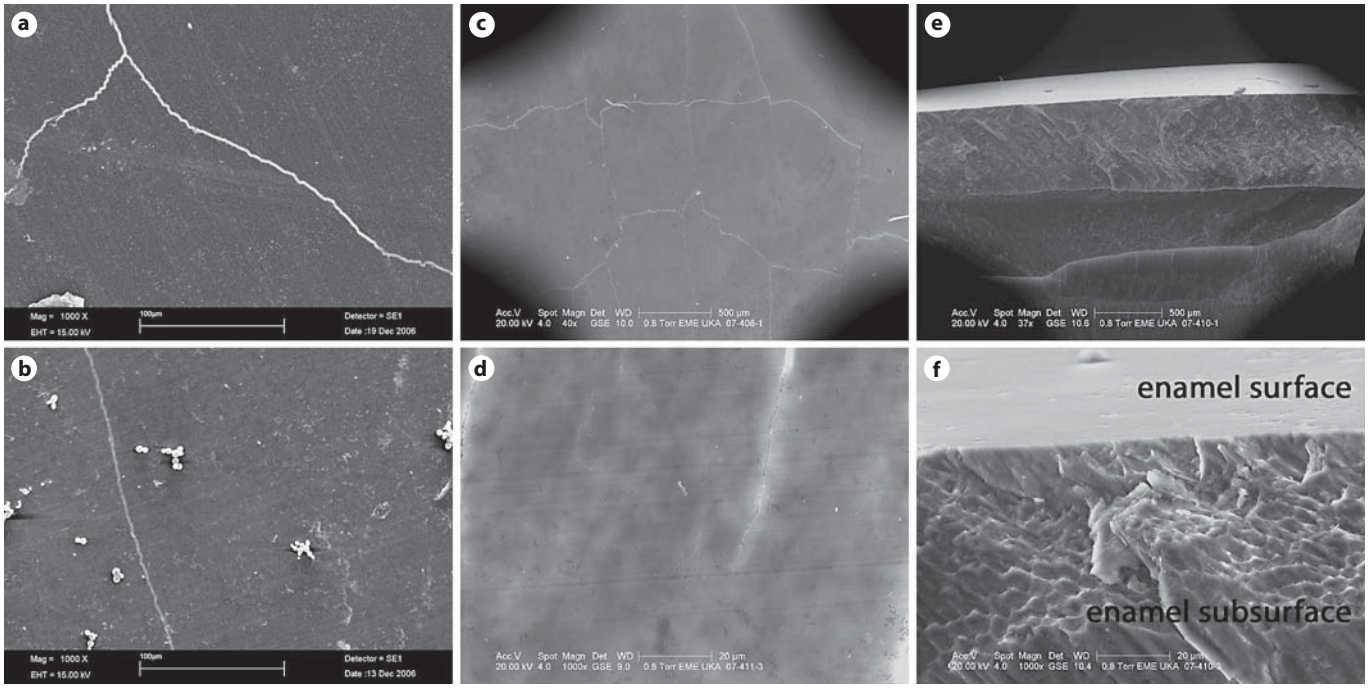


Fig. 2. Scanning electron micrographs of the sample surfaces from the control group and from the laser groups causing the greatest inhibition of caries progression. **a** Control group. **b** Group laser 0.3 J/cm^2 (10 μs, 154 Hz). **c**, **d** Group laser 9 s (0.3 J/cm^2, 5 μs, 226 Hz) at lower and higher magnifications, respectively. **e**, **f** Cross-sectional scanning electron micrographs of group laser 9 s at lower and higher magnifications, respectively. No signs of ablation, melting or damage, such as cracks, can be detected on any of the surfaces (irradiated and control) and cross-sectional images. Especially in **f** the interface surface/subsurface of the irradiated sample can be observed.

solution inhibition. This inhibition was also statistically significantly higher than that of fluoride treatment (25%). Furthermore, this result was obtained with an energy density of only 0.3 J/cm^2 and a pulse duration of 5 μs. A search in the scientific databases showed that this preventive effect of enamel irradiation with a CO_2 laser (λ = 10.6 μm) had previously been demonstrated only for higher energy densities (2.5–83.3 J/cm^2) and much longer (100–50,000 μs) or much shorter (0.1–0.2 μs) pulse durations [Esteves-Oliveira et al., 2008; Featherstone et al., 1998; Klein et al., 2005; Nelson et al., 1986; Steiner-Oliveira et al., 2006; Tagliaferro et al., 2007; Tsai et al., 2002]. In this context, it is important to note that low energy densities and short pulse durations are said to cause less thermal damage to the surface and less irreversible pulp inflammation, which can impair in vivo application [Fried et al., 1996].

Only two studies were found that apparently demonstrated demineralisation inhibition after irradiation with a CO_2 laser (λ = 10.6 μm) at 0.3 J/cm^2 [Hsu et al., 2000, 2001]. However, assuming that the data on average power, repetition rate and beam diameter were correctly reported by the authors, the energy density was 3.4 J/cm^2 and not 0.3 J/cm^2 as mentioned in the publication. This may be the reason why high inhibition (99%) was found.

In contrast to what was initially expected, for the pulse duration of 10 μs in the first step of the present experiments, the lower energy densities tested (0.1 and 0.3 J/cm^2) were the ones which caused the greatest reduction in lesion progression. Featherstone et al. [1998] described an opposite relationship between energy density and observed effects when studying pulses of 100 μs. The authors observed the higher percentages of caries inhibition with the higher energy densities, considering an interval between 2.5 and 12.5 J/cm^2. The reason for this different energy density/effect relationship could be the differences in the way the laser parameters were defined. In the present investigation, the principal limitation when selecting the parameters was the operating mode of the laser equipment. The device only offers the possibility of choosing the values for the on and off times (duty cycle) of the laser device. This meant, for example, that the rep-

etition rates necessarily had to be different in the groups, in order to maintain the initially predefined condition of having the same pulse duration in all groups (experiment 1). The other preconditions restricting the irradiation parameters were: (1) it was to be possible to obtain 5 different energy densities for the same pulse duration; (2) the average power was to have the same order of magnitude; (3) there was to be no visible sign of enamel ablation or carbonisation at the surface. Therefore, for some irradiation conditions, namely the ones with higher energy densities or higher repetition rates, the irradiation time had to be reduced to ensure no surface ablation or carbonisation.

It is important to note that the number of overlapping pulses was higher in the groups with the greatest caries inhibition. These results are in accordance with the results of Kantorowitz et al. [1998], who, after enamel irradiation with a CO_2 laser (λ = 10.6 μm) with different numbers of overlapping pulses (1, 5, 25 and 100), observed greater inhibition of enamel mineral loss at increasing numbers of overlapping pulses. The results varied between 48 and 87% inhibition and the effect stabilised between 25 and 100 pulses, where there was no significant difference between the two conditions [Kantorowitz et al., 1998].

Moreover, the elimination of carbonate from enamel apatite increases with rising numbers of overlapping pulses. For irradiation with pulses of 2 μs (λ = 10.6 μm), a reduction of 100% in the carbonate content at the enamel surface was obtained with 5 overlapping pulses, whereas only 60% was obtained with 1 pulse [Fried et al., 1999]. There is thus evidence that, for subablative irradiation, the overlapping of a higher number of pulses on the same area has the potential to increase the resistance of enamel to demineralisation, until a threshold of stabilisation is reached.

For the irradiation conditions tested in the present study, the morphological observations showed that common side effects of laser irradiation were not observed on the enamel surface. Side effects like cracks, ablation and carbonisation have previously been reported [Fried et al., 1996, 2001; McCormack et al., 1995], and their presence can compromise in vivo application.

Among the possible models for simulating caries in vitro, the pH-cycling model delivers results more comparable to clinical outcomes. Use of this model allows not only simulation of the demineralisation caused by the acids produced in dental plaque, but also the remineralisation process [Featherstone, 1996]. As regards the substrate used, since many studies have used bovine teeth for caries simulation in vitro, this substrate was chosen for the present study. The advantages of using bovine teeth are the possibility of obtaining bigger areas of plane enamel and their better availability compared to human enamel. Besides, these teeth present composition and optical properties similar to human teeth, and the mechanism of caries formation is also the same [Arends et al., 1980; Bachmann et al., 2003; Borggreven et al., 1980; Fried et al., 1997]. However, the fact that in these teeth the rate of demineralisation progression is higher [Featherstone and Mellberg, 1981] must be taken into consideration.

Although in vitro studies are adequate for the evaluation of caries-preventive methods, only in situ and in vivo studies are capable of offering the clinical evidence necessary to prove the efficacy of a therapy. Therefore, although 81% caries inhibition could be achieved in the present study, the parameters that caused this reduction still need to be further tested before clinical application.

Moreover, the safety of the irradiation conditions for the pulp must be ensured. For this reason, the parameters used in this study were also tested in a three-dimensional finite element model, the results showing that the temperature rise 2.8 mm below the surface was less than 3°C [Esteves-Oliveira et al., 2007]. Nevertheless, further detailed temperature investigations are clearly necessary in a next step.

In conclusion, the optimum CO_2 laser (λ = 10.6 μm) parameters obtained in the present investigation (0.3 J/cm^2, 5 μs, 226 Hz and 2,036 overlapping pulses) were able to decrease enamel caries progression by 81% without causing surface and subsurface thermal damage observable by scanning electron microscopy.

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